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MANIPULATING AND ENHANCING CITRUS FRUIT RESISTANCE TO THE CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The natural resistance of citrus fruit to attack by the Caribbean fruit fly (caribfly) varies according to fruit senescence and cultivar. Lemons are virtually immune, oranges are highly resistant, and grapefruit are initially somewhat resistant, at least before becoming senescent. A method allowing an extension of the innate early-season resistance of grapefruit to fruit flies is described. Elucidation of lemon immunity factors is needed so that the responsible factors can be incorporated into less resistant cultivars, perhaps through use of genetic engineering techniques. By extending and/or increasing resistance of grapefruit, it may be possible to achieve adequate prevention of caribfly infestation throughout most of the season without the need to use expensive and troublesome postharvest disinfestation treatments in order to ship the fruit to quarantine-sensitive states and countries.

RESUMEN

La resistencia natural de las frutas de cítricos al ataque de la mosca de la fruta del caribe (Caribfly) varía de acuerdo a la edad de la fruta y a el tipo de cultivar. Los limones son practicamente immunes, las naranjas son muy resistentes, las toronjas son mas resistentes al ser recien cosechadas, pero luego se veuelven menos resistentes. Se describe un metodo para alargar el periodo de resistencia a la mosca en estas toronjas recién cosechadas. Se necesita identificar los factores que confieren imunidad a el limón, para asi incorporar estos factores en los cultivares poco resistentes, talvez mediante el uso de ingeniería genética. Al extender o al incrementar la resistencia a la toronja, puede ser posible el alcanzar una prevención contra la infestación de la mosca de la fruta

a lo largo de todo el período de cosecha, lo cual disminuiría la necesidad de los tratamientos postcosecha, y ayudaría a facilitar el envío de fruta a paises o estados con reglamentos cuarentenarios para este problema.

Many changes have occurred in the procedures used to assure the absence of the Caribbean fruit fly (caribfly), *Anastrepha suspensa* (Loew), from Florida grapefuit since the 1984 Environmental Protection Agency ban on use of ethylene dibromide (EDB) for postharvest fumigation (Greany & Riherd 1993). Formerly, most grapefruit destined for shipment to Japan, California, Arizona, and Texas were disinfested after harvest; now, the emphasis is instead on developing biorational, cost-effective methods to prevent field infestation of grapefruit by the caribfly. A variety of methods are being employed to reduce the likelihood of infestation, including removal of alternate host plants, use of malathion bait sprays, and reliance on early season resistance of grapefruit to attack (Simpson 1993).

We believe it may be possible to take further advantage of the inherent resistance of citrus fruit to caribfly attack early in the season (Greany 1989, Greany et al. 1983, 1985) by delaying fruit senescence. Ultimately, by identifying factors in lemons that confer immunity to fruit flies (Greany 1993), it might be possible to incorporate these into other less resistant cultivars through plant breeding or by genetic engineering.

Extending Early Season Resistance

One possibility for manipulation of fruit fly susceptibility is to use the naturally-occurring plant growth regulator gibberellic acid (GA_3) to extend the early season resistance routinely exhibited by grapefruit. Research has shown that GA_3 can serve as a "status quo" hormone, delaying senescence of citrus fruit peel. The traditional use of GA_3 is to allow treated fruit to be kept longer after harvest without suffering the usual amount of postharvest decay (Coggins 1973, Ali Dinar et al. 1976, Ferguson et al. 1982, Lima & Davies 1984, McDonald et al. 1987, El-Otmani & Coggins 1991). Subsequent research has shown that GA_3 treatment also reduces susceptibility of citrus fruit to fruit flies (Greany et al. 1987, McDonald et al. 1988, Rössler & Greany 1990, Greany et al. 1991). To achieve this, citrus growers must treat the fruit while still on the tree and still externally green (i.e., prior to "colorbreak"). This causes the fruit peel to remain green and tough, but it does not interfere with internal fruit ripening. If desired, treated fruit can be "degreened" prior to sale by use of another natural plant hormone, ethylene, which causes the chlorophyll to be broken down, revealing the orange or yellow color normally associated with ripe oranges or grapefruit (McDonald et al. 1987). In nearly 30 years of use (Coggins & Lewis 1965), no harm to humans has ever been found due to exogenous application of GA_3 to citrus fruit to delay senescence and prolong the harvest season. Studies of metabolism of GA_3 in the fruit revealed that GA_3 is absorbed as, and remains primarily in, the applied form (Ferguson et al. 1986).

Our goal is to use GA_3 treatments to reduce the need to use conventional insecticides to assure the absence of caribfly from grapefruit. Because of increasing concern for the environment, there is a possibility that malathion bait sprays may be banned, as was the use of EDB fumigation in 1984. This could jeopardize the annual shipment of $100-200 million worth of grapefruit from Florida to Japan, California, Arizona, and Texas where the caribfly could pose a threat if introduced and established (Riherd 1993, Greany & Riherd 1993, Simpson 1993). Postharvest treatments based on chilling or heating are available or are being developed (Sharp 1993, McDonald et al. 1993), but are not preferred by growers and shippers as compared to field control strategies. Methyl bromide fumigation is routinely used as a postharvest treatment of citrus fruit being shipped to California, Arizona and Texas, but its continued use is threatened due to concern for its potential harm to the ozone layer.

By using GA_3, it may be possible to delay application of malathion bait sprays. Research has shown that GA_3-treated fruit are less attractive to caribfly and are less susceptible even if attacked (Greany et al. 1987). Results of field studies in Florida in 1991 and 1992 showed an 80% reduction of caribfly attack on grapefruit as a result of GA_3 treatment (Greany et al. unpub. data). In addition to protecting the fruit, use of GA_3 treatments instead of conventional insecticides should protect natural enemies important to biological control of other citrus pests such as scale insects (Rössler & Greany 1990).

Our current research is intended to prove the efficacy of GA_3 treatments in the field and to reduce the cost of the GA_3 treatments through optimization of treatment timing and formulation of the active agent and the associated surfactant needed to facilitate penetration of the hormone into the fruit peel. In addition, we are developing quantitative indices of fruit fly susceptibility upon which objective judgements could be made whether to allow shipment of grapefruit without postharvest treatment. In this regard, we have correlated fruit susceptibility with 4 senescence features: peel color, resistance to puncture, oil content, and limonin content (Greany et al. in preparation, Shaw et al. 1991, Wilson et al. 1990). Finally, we are evaluating the relative susceptibility of GA_3-treated vs. untreated grapefruit to harm associated with the use of postharvest hot air treatments (McDonald et al. 1993). Preliminary studies by R. McDonald and Wm. Miller (USDA ARS, Orlando, FL, unpub. data) suggest that GA_3-treated grapefruit are less easily damaged by heat treatment.

Genetic Transfer of Resistance Factors

A more enduring (and more distant) approach to the control of tephritids would be to mobilize natural resistance of closely related plants through either conventional breeding or genetic engineering. The latter possibility is rapidly approaching fruition through advances in tissue culture and molecular biology. Besides advances in these areas, however, genetic engineering requires fundamental knowledge of the biochemistry behind resistance mechanisms. Since mechanisms involving more than a single primary gene product (i.e. the enzyme directly responsible for resistance, or responsible for synthesis of a compound which confers resistance) are presently too complex to consider, we are searching for sources of single-gene resistance.

A potential source of single-gene resistance in lemons and limes has been suggested to us (T. Waiss, USDA ARS, Albany, CA, pers. comm.). These fruits have demonstrated a virtual immunity to attack by the caribfly (Nguyen & Fraser, 1989, Hennessey et al. 1991). This resistance in lemons and limes strongly correlates with content of a specific flavonoid, the flavanone eriodictyol, and its glycosides eriocitrin and neoeriocitrin (Horowitz & Gentili, 1960, Albach and Redman 1969, Kamiya et al. 1979). Furthermore, eriodictyol itself has shown strong growth inhibition toward *Heliocoverpa zea*, while its precursor, naringenin (present in peel of most citrus fruits), showed no activity (Elliger et al. 1980). If the same holds true for the caribfly, we may have a unique opportunity to genetically engineer natural resistance into citrus through transfer of a single gene.

Flavonoids undergo numerous metabolic conversions, many of them enzymatically mediated by cytochrome P-450 monooxygenases found in the endoplasmic reticulum (microsomes) of plant cells (Heller & Forkmann 1988). The advantages of P-450-mediated single-step hydroxylation of naringenin to eriodictyol are apparent. Only one enzyme is involved, and that enzyme, microsomal flavanone 3'-hydroxylase, has been prepared and characterized from several plant species (Heller & Forkmann 1988). All described citrus cultivars contain appreciable pools of naringenin and one of its glycosides, naringen. Recently, we demonstrated naringenin hydroxylase activity in undifferentiated callus tissue from oranges (JS, T. Burke, & R. Mayer, USDA ARS, Orlando, FL, unpub. data). Further characterization of the enzyme, especially by isolation, partial amino acid sequencing, and immunochemical comparison with other plant cytochrome P-450s, will allow us to identify and isolate the gene that codes for it. The close phylogenetic relationships of citrus cultivars within the Rutaceae may enhance the probability of successful genetic transfer, gene expression, and enzymatic activity.

CONCLUSIONS

Clearly, the future of crop plant development lies in our ability to alter selected physiological and biochemical characteristics of a crop. Since the advent of agricultural biotechnology, whether we attempt to produce a plant with improved defense mechanisms, enhanced food quality, or increased productivity, our capabilities are limited only by the depth of our understanding of underlying physiological mechanisms. We have presented a brief overview of two distinct and progressive approaches to increasing the defenses of citrus fruit against tephritid fruit flies, one near term, the other long term. Both approaches derive from an increased understanding of physiological processes in the fruit and their influence on fruit fly attack and development. Continuing emphasis on gaining and applying basic knowledge should enable ever more targeted and successful instances of biorational pest management through host plant resistance.

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FUTURE DIRECTIONS IN CONTROL OF THE CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The Caribbean fruit fly (caribfly), *Anastrepha suspensa*, was introduced into Florida in 1965 and has spread throughout the southern portion of the state, infesting species of tropical and subtropical fruits. Although it does not present a threat to citrus production, it has become a quarantine pest of citrus fruits. Several novel methods of control are presented that fall under the 3 categories: detection, exclusion, and control/eradication. Research directed toward better lures and traps are underway. Exclusivity is being addressed by making the fruit unattractive for oviposition or by adding antibiotic factors to the host fruit. The concept of the fly-free zone is supported by control/eradication technology. Bait sprays that contain malathion may be phased out in the future. Replacement for this and other chemicals must be considered for future control and in support of the sterile male release method.

RESUMEN

La mosca de la fruta del Caribe (Caribfly), fue introducida en Florida en 1965 y se ha expandido en la porción sur del estado infestando especies de frutas tropicales y subtropicales. Aunque no representa una amenaza a la producción de cítricos, se ha convertido en una plaga cuarentenaria de cítricos. Los metodos de control se pueden agrupar en tres categorias: detección, exclusión y control/erradicación. La investigacion dirigida hacia mejores atrayentes y trampas se esta realizando. Se efectua exclusión al hacer esta fruta menos atractiva para oviposición o al agregar antibioticos a la fruta hospedera. El concepto de una zona libre de moscas se basa en una tecnologia de control/erradicación. Las asperciones con cebos que contengan malathion pueden dejar de utilizarse en un futuro. El reemplazo de estos y otros quimicos puede ser considerado para un control futuro y en favor del uso de liberaciones de machos esteriles.